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A new species of *Braunsia* (Hymenoptera, Braconidae, Agathidinae), a natural enemy of *Cydalima perspectalis* (Lepidoptera, Crambidae) from South Korea: species description and notes on its biology

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Abstract

The invasion of *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae) poses a significant threat to European ecosystems and North American ornamentals. In an effort to identify potential biological control agents from the native habitats of *C. perspectalis*, a field survey was conducted from 2022 to 2024 in South Korea. During the survey, a new braconid agathidine species of *Braunsia* Kriechbaumer, 1894 was discovered: *Braunsia hodorii* Kang, **sp. nov.** The new species is delimited and described based on morphological, molecular, and phylogenetic data. Additionally, information on the behavior of *B. hodorii* **sp. nov.** is presented regarding the larval external feeding phase. A key to species of *Braunsia* in Korea is included accompanied by detailed images. This research contributes to the evaluation of natural enemies as biological control agents against *C. perspectalis* in its invasive range.

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Keywords
Box tree moth, braconid taxonomy, classical biological control, hymenopteran parasitoid, invasive species

Introduction

The expansion of global trade has played a significant role in the introduction of non-indigenous species, particularly through commerce of live plants (Meyerson and Mooney 2007; Roques et al. 2016). Along with the trade of ornamental box trees (also called boxwood, *Buxus* spp., Buxaceae) from Asia to Europe, a lepidopteran insect pest, the box tree moth, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae), was accidentally introduced to European countries (Kenis et al. 2013). Since its first record in southwestern Germany in 2007 (Krüger 2008), the moth has been expanding its range across Europe and into West Asia (Kenis 2015; Bras et al. 2019) and North Africa (Haddad et al. 2020). The widespread expansion of the box tree moth has had devastating effects on the common box tree (*Buxus sempervirens* L., 1753) (Wan et al. 2014; Mitchell et al. 2018), which has been valued for ornamental purposes in Europe since the Roman period (van der Veen et al. 2009). In North America, *C. perspectalis* was reported the first time in Ontario, Canada, in 2018 (NAPPO 2019; Wiesner et al. 2021) and also detected in Connecticut, Michigan, Ohio, and South Carolina, USA, in 2021 (USDA APHIS 2021). In 2014, boxwood comprised 15 percent of broadleaf evergreen sales in the United States, with an estimated value of 126 million US dollars (USDA-NASS 2015), so the potential impact is considerable.

For the development of classical biological control strategies to mitigate the spread and impact of *C. perspectalis*, the foreign exploration of potential biological control agents was initiated in the native ranges of *C. perspectalis* in Asia (Wan et al. 2014). The native distributions of *C. perspectalis* spans across the east palearctic and oriental regions, including China (Walker 1859), Far East Russia (Kirpichnikova 2005), India (Hampson 1896), Japan (Inoue 1982), and South Korea (Gu 1970). A total of 11 species of arthropod predators and parasitoids have been recorded from China, Japan, and Switzerland (Wan et al. 2014). These natural enemies are in need of more detailed study due to identification errors and incomplete or erroneous biological data of the parasitoids of *C. perspectalis.* Discovering host-specific parasitoids on *C. perspectalis* is crucial to identify potential classical biological control agents. Thus, enhancing our understanding of the interactions between parasitoids and their hosts is imperative for developing more targeted and efficient pest management strategies.

CABI and a research team of the Kyungpook National University collaborated and searched for natural enemies of *C. perspectalis* in South Korea. Among the hymenopteran parasitoids that were found during the surveys, a new species of *Braunsia* Kriechbaumer, 1894 (Agathidinae Haliday, 1833) was discovered. Most members of Agathidinae are known to be solitary koinobiont endoparasitoids of leaf- rolling or stem-boring lepidopteran larvae (Sharkey 1996). *Braunsia* is an Old World genus, and members are recorded as parasitoids of Erebidae, Lasiocampidae, Noctuidae, and Pyralidae (Yu et al. 2016). Seventy-three species of *Braunsia* were recorded worldwide

prior to the current project (Yu et al. 2016; Tang et al. 2017), and only two species were recorded from Korea (Park and Lee 2022).

During a three-year survey period (2022 to 2024), members of the new species of *Braunsia*, *Braunsia hodorii* sp. nov., were reared from larvae of *C. perspectalis* and its biology was recorded with detailed images and videos. A description of *Braunsia hodorii*, host and biological information, DNA data, a phylogenetic tree based on molecular data, and a key to species of *Braunsia* of Korea are included here.

Material and methods

Collecting and rearing

Larvae of *C. perspectalis* were collected from wild *Buxus* sp. at two sites: Munsan-ri, Yeongwol-eup, Yeongwol-gun, Gangwon-do, South Korea (37°15'48.4"N, 128°31'13.8"E) in May 2022 and 2023 and Bangjeol-ri, Yeongwol-eup, Yeongwol-gun, Gangwon-do, South Korea (37°12'20.9"N, 128°26'1.2"E) in May 2022 and 2024. The collected larvae of *C. perspectalis* were reared in groups of approximately 20 larvae with similar body length and weight on commercial *Buxus* spp. plants (Keewoom farm, Okcheon-gun, Chungcheongbuk-do, South Korea). This rearing was conducted during May–June 2022, utilizing plastic pots (28.0 cm diameter × 28.0 cm height, 10 L; IKEA) with 4 L of potting soil mix (Baroker bed soil, Seoul Bio., Eumseong-gun, Chungcheongbuk-do, South Korea) and 1 L pumice stone (Hwabun world, Chilgok-gun, Gyeongsangbuk-do, South Korea). In May–June 2023, collected larvae were reared in plastic cages (7.2 cm wide × 7.2 cm length × 10.0 cm height, Insect breeding box, SPL Life Science, Pocheon-si, Gyeonggi-do, South Korea). Each cage contained commercial *Buxus* spp. branches with leaves with 5 larvae per cage. Both the pots and plastic cages were kept in a laboratory at Kyungpook National University in Daegu, South Korea under controlled conditions of 25 ± 1 °C and 56 ± 12% relative humidity during May–June 2022 and May–June 2023.

When parasitoid larvae emerged from the *C. perspectalis* larvae, the behavior of the parasitoids were observed until they became adults. Images and videos were taken and recorded. Adult parasitoids were transferred to a rearing cage (10.0 cm diameter × 4.0 cm height; Insect Breeding Dish & Jar, SPL Life Science, Pocheon-si, Gyeonggi-do, South Korea). A 10% honey water solution (Australia Eucalyptus Honey, Capilano Honey Limited, Queensland, Australia) was provided as often as needed. Dead specimens were preserved in a 1.5 mL microcentrifuge tube with 99% ethanol.

Specimen information

Type specimens are deposited in the insect collection in the Department of Entomology of Kyungpook National University (**KNUS**; Sangju-si, Gyeongsangbuk-do, South Korea) and will be deposited in the National Institute of Biological Resources (**NIBR**; Incheon, South Korea). Non-type specimens are deposited at **CABI** (Delémont, Switzerland).

Morphological analysis

Using stereo microscopes (Leica M165 C & Leica S9E, Leica Microsystems, Wetzlar, Germany), braconid specimens were examined. Identification keys, diagnostic characters, species descriptions, checklists, and phylogenetic data by Sharkey (1996), Sharkey and Bennett (2004), Sharkey and Clutts (2011), Tang et al. (2017), and Park and Lee (2022) were used for morphological identifications. Images were captured with a Sony A7R4 camera (Sony, Japan), equipped with a Canon Macro Photo Lens MP-E 65 mm f/2.8 1–5 × (Canon, Japan). Images were stacked using Zerene Stacker (Zerene System, USA) and were then edited using Adobe Photoshop 2024 (Adobe, USA). The same Adobe software was used to measure morphometric characters. The external morphological terminology is based on Shakey and Wharton (1997), and a modified Comstock-Needham system for wing veins by Sharkey and Wharton (1997) was used. Terms for external body sculpture are based on Harris (1979). The following are acronyms used throughout the article: POL: distance between posterior ocelli, T1: first metasomal tergite, T2: second metasomal tergite, T3: third metasomal tergite, T4: fourth metasomal tergite, T5: fifth metasomal tergite, T6: sixth metasomal tergite.

Molecular analysis

Following manufacturer's instructions, genomic DNA was extracted using a PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Legs or whole-body samples were macerated using a pestle in 180 µL of genomic digestion buffer and 20 µL of proteinase K (50 µg/mL). All extracted DNA samples were stored at -20 °C until further analysis. PCR amplification was performed using Veriti™ 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, Boston, Massachusetts, USA). PCR reaction volumes were 30 µL comprising of 15 µL of SolgTM 2 × Taq Pre-Mix (Solgent, Daejeon, South Korea), 2 µL of each primer (10 pmol/µL), 2 µL of DNA template and 9 µL of double-distilled H_2O in a total reaction. Approximately 650 bp of the mitochondrial cytochrome *c* oxidase subunit I (COI) DNA region was targeted and amplified using the following primer sets:

LCO1490 (5′ - GGTCAACAAATCATAAAGATATTGG - 3′) and
HCO2198 (5′- TAAACTTCAGGGTGACCAAAAAATCA- 3′) (Folmer et al. 1994),
or LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and
LepR1 (5′-TAAACTTCTGGATGTCCAAAAAATC A-3′) (Hebert et al. 2004).

The PCR conditions for COI included an initial denaturation at 94 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 30 secs, annealing at 50 °C for 40 secs, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 mins. The D2 region of nuclear 28S rRNA (28S) genes was amplified using the primer set:

28SD2hymF (5′ - AGAGAGAGTTCAAGAGTACGTG - 3′) (Belshaw and Quicke 1997) and 28SD3hymR (5′ - TAGTTCACCATCTTTCGGGTC - 3′) (Belshaw et al. 2001).

The 28S PCR conditions followed Smith et al. (2008) but the number of cycles reduced from 35 to 30; consisted of initial denaturation at 94 °C for 2 mins, 30 cycles of denaturation at 94 °C for 30 secs, annealing at 56 °C for 30 secs, extension at 72 °C for 2 mins, and a final extension at 72 °C for 2 mins. The PCR products were separated by 1% agarose gel electrophoresis, stained with ethidium bromide solution, and bands were visualized under ultraviolet light. Crude PCR products were cleaned up using Solg™ Gel & PCR Purification Kit (Solgent, Daejeon, South Korea) and sequenced at the Solgent Sequencing Facility (Solgent, Daejeon, South Korea).

DNA assembly and phylogenetic analyses

Both COI and 28S DNA sequences produced by Sanger sequencing were edited and assembled in Geneious Prime 2024.0.2 (https://www.geneious.com). To construct reliable phylogenetic trees, COI sequences from *Aerophilus* Szépligeti, 1902 and *Braunsia* species deposited in the Barcode of Life Datasystem (BOLD) (Hebert et al. 2003), along with 28S DNA sequences from GenBank of the National Center for Biotechnology Information (NCBI) were downloaded and included into the current project (Table 1). All obtained sequences were aligned using MUltiple Sequence Comparison (MUSCLE) (Edgar 2004) in GENEIOUS PRIME. For the genetic distance estimation, MEGA11 was used, and the Kimura-2-parameter model (K2P) was selected (Kimura 1980). A concatenated 28S+COI dataset was produced using MEGA11 (Tamura et al. 2021). The concatenated 28S+COI dataset was partitioned by codon position for COI (1st, 2nd, and 3rd codon positions; 3 partitions) and codon position and gene region for 28S+COI (gene region, 1st, 2nd, and 3rd codon; 4 partitions). Neighbor joining (NJ) analyses was conducted using PAUP* (v. 4.0; Swofford 2003) with the p-distance setting. For Maximum Likelihood (ML) analyses, RAxML version 8.2.11 (Stamatakis 2014) was utilized with a bootstrapping strategy involving 1,000 Rapid replicates to gauge the reliability of the ML tree. Model selection was facilitated by the FindModel tool (http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html), resulting in the adoption of the GTR+G rate model—General Time Reversible with gamma-distributed rate variation among sites. The choice of this model is informed by the principles outlined in the MODELTEST paper (Posada and Crandall 1998), which is the basis for the FindModel approach in ML analyses. Bayesian inference analyses (BI) for the concatenated dataset was conducted using MrBayes (v.3.2.6; Ronquist et al. 2012) with the GTR+G rate model. The analysis involved conducting Markov Chain Monte Carlo (MCMC) searches, including two independent runs of 50,000,000 generations, with four chains and sampling every 1000 generations. The first 50% of the generations were discarded as burn-in. To enable individual partitions to possess their unique collection of parameter approximations, the following parameters were unlinked: statefreq, revmat, shape, tratio, and pinvar. *Aerophilus jdherndoni* and *A. minys* were selected as outgroups because it was shown to be the sister-group to *Braunsia* (Sharkey and Chapman 2017, 2018).

Table 1. Metadata of specimens used for phylogenetic analyses.

Metadata Species	NCBI		BOLD		Locality	Voucher Number
	COI	28S	COI	28S		
Aerophilus jdherndoni	–	KU059013	AAU4708	–	USA	H1307
Aerophilus minys	–	KU059031	AAP1178	–	USA	H1263
Braunsia fumipennis	MF098378	HQ667946	AAV0736	–	Thailand	H915
Braunsia smithii	–	HQ667949	AAV0735	–	Thailand	H906
***Braunsia* sp.**	–	KU058998	AAP3216	–	Republic Congo	H1876
***Braunsia* sp.**	–	KU058999	AAV0730	–	Uganda	H1893
***Braunsia* sp.**	–	KU058996	AAV0732	–	Kenya	H1884
***Braunsia* sp.**	–	KP943695	AAV0733	–	Republic Congo	H1891
***Braunsia* sp.**	–	KU058992	AAV0734	–	Kenya	H1892
***Braunsia hodorii* sp. nov.**	PP437200	PP464023	–	–	Korea	P7_2
***Braunsia hodorii* sp. nov.**	PP441967	PP464024	–	–	Korea	P7_6

Results

Species delimitation

Based on morphological characteristics, we hypothesize that *Braunsia antefurcalis*, *B. matsumurai*, and *B. hodorii* sp. nov. are distinct species. COI genetic distances among *Braunsia* taxa ranged from ~4.7% to ~12.7% (Table 2). *Braunsia hodorii* sp. nov. sequences were ~7.7% different from the nearest neighbor. *Braunsia hodorii* sp. nov. was also clearly recovered as distinct in phylogenetic data based on 28S+COI (Fig. 1) data.

Phylogenetic relationships

Both BI and ML phylogenetic trees recovered identical topologies. In contrast, the NJ trees based on 28S + COI and COI only data showed differences in the main clusters (Suppl. materials 1, 3). Most clades exhibited robust nodal support values in ML and BI trees based on 28S + COI data, but relatively not in clusters in the NJ trees (Suppl. materials 1, 3), and a clade consisting of *Braunsia* sp. Republic Congo H1876, *Braunsia* sp. Uganda H1893,

Table 2. Estimates of genetic distances between COI sequences (rounded up to 3 digits).

	***Braunsia* sp. H1891**	***Braunsia* sp. H1892**	***Braunsia* sp. H1876**	***Braunsia* sp. H1884**	***Braunsia* sp. H1893**	***Braunsia fumipennis* H915**	***Braunsia smithii* H906**	***Braunsia hodorii* sp. nov. P7_2**	***Braunsia hodorii* sp. nov. P7_6**
***Braunsia* sp. H1891**									
***Braunsia* sp. H1892**	0.047								
***Braunsia* sp. H1876**	0.088	0.077							
***Braunsia* sp. H1884**	0.081	0.064	0.062						
***Braunsia* sp. H1893**	0.079	0.080	0.055	0.071					
***Braunsia fumipennis* H915**	0.110	0.093	0.102	0.104	0.089				
***Braunsia smithii* H906**	0.125	0.114	0.101	0.101	0.113	0.097			
***Braunsia hodorii* sp. nov. P7_2**	0.127	0.103	0.100	0.096	0.091	0.079	0.077		
***Braunsia hodorii* sp. nov. P7_6**	0.127	0.103	0.100	0.096	0.091	0.079	0.077	0.000	

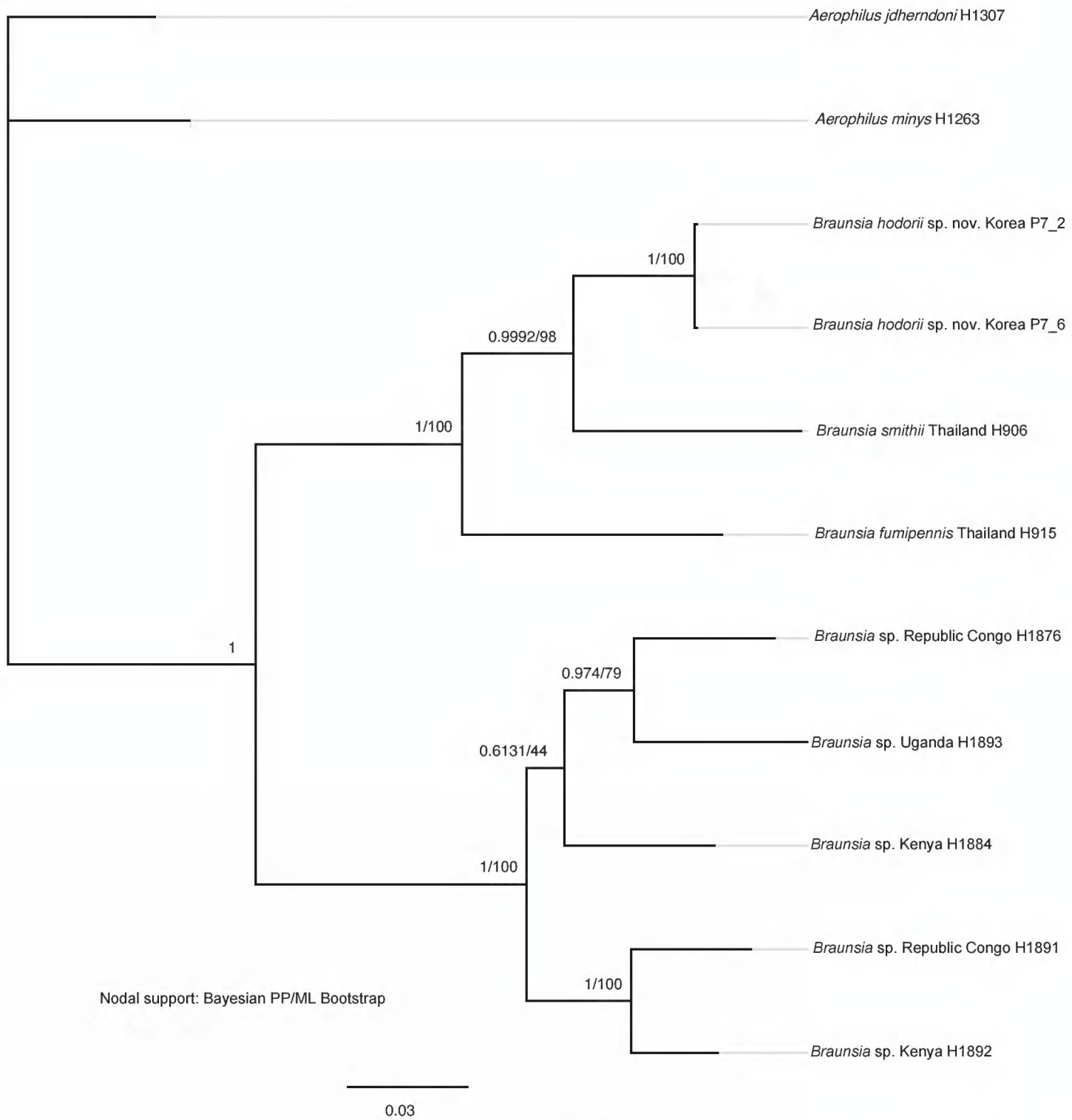


Figure 1. The BI tree based on concatenated 28S and COI data for *Braunsia* species. Bayesian posterior probabilities and Maximum Likelihood bootstrap values are plotted.

and *Braunsia* sp. Kenya H1884 in the ML and BI trees (Fig. 1 and Suppl. material 2). For *Braunsia*, two main clades were recovered in the ML and BI trees. One clade consists of three species occurring in the Palearctic and Oriental regions: *B. hodorii* sp. nov., *B. smithii* (Dalla Torre, 1898), and *B. fumipennis* (Cameron, 1899). The remaining five undescribed species occur in the Afrotropical region, forming the second clade. Within the Palearctic and Oriental clade, *B. fumipennis* was recovered as the sister taxon to *B. hodorii* sp. nov.

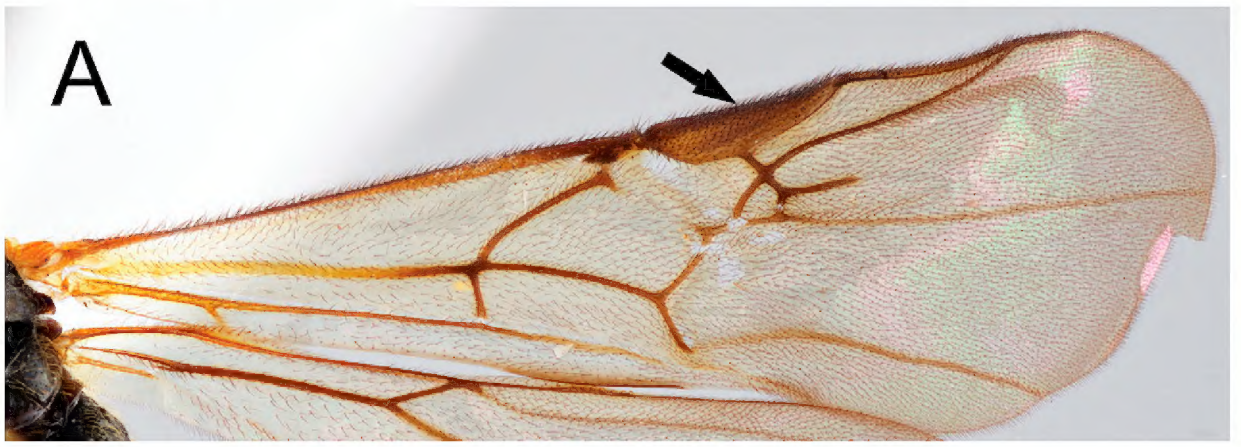
Systematics

Braunsia Kriechbaumer, 1894

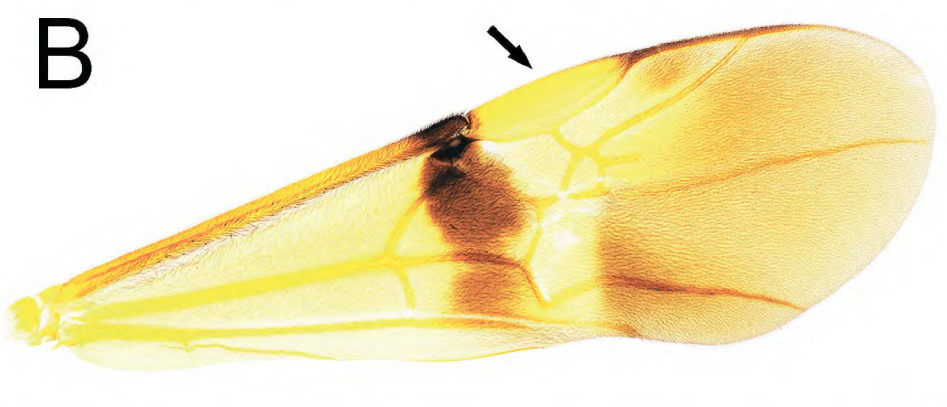
Type species. *Braunsia bicolor* Kriechbaumer, 1894, designated by Viereck (1914).

Key to species of *Braunsia* of Korea

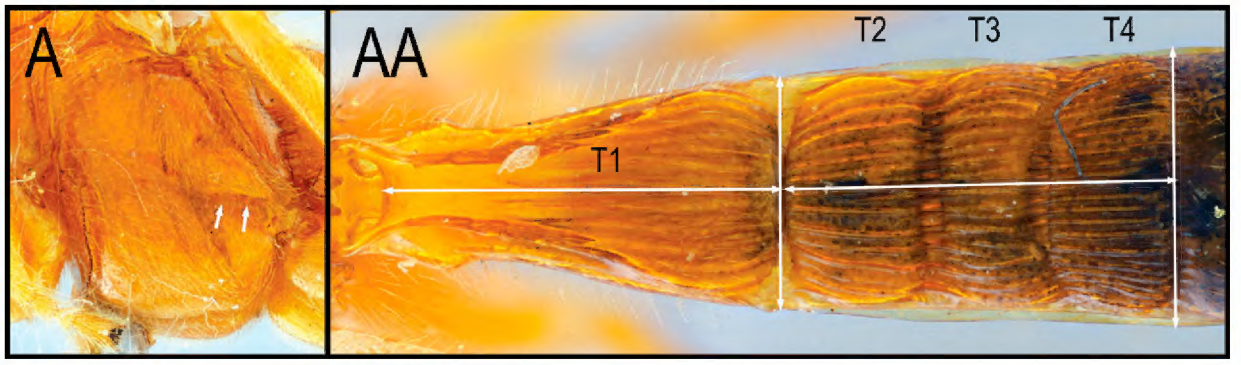
1 **A.** Forewing with entirely dark brown pterostigma .....
.....***B. antefurcalis*** **Watanabe, 1937**



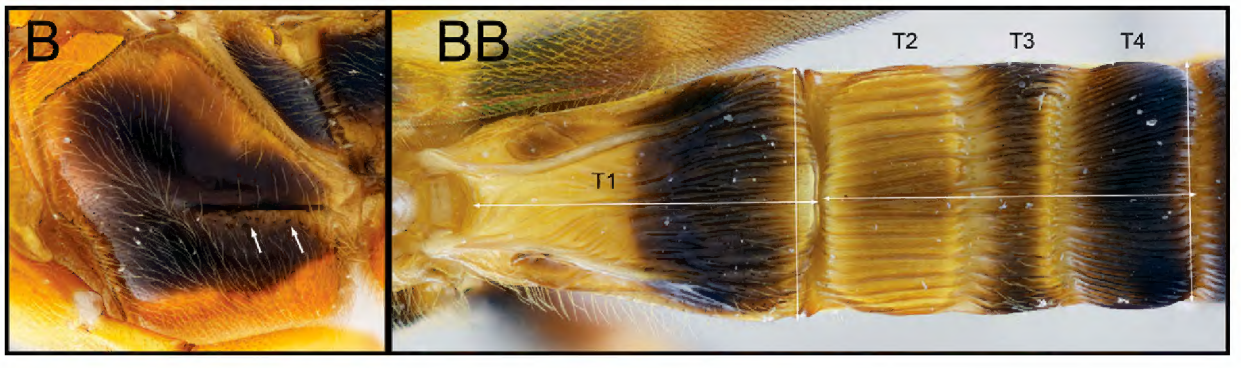
– **B.** Forewing with partially or completely pale pterostigma.....**2**



2 **A.** Mesopleuron entirely pale; precoxal sulcus less crenulate; **AA.** T1 uniformly colored, entirely melanic or pale, 1.7 × longer than apical width; T2–T4 1.4 × longer than apical width .....***B. matsumurai*** **Watanabe, 1937**



– **B.** Mesopleuron mostly black; precoxal sulcus more crenulate; **BB.** T1 bicolored, melanic at posterior half, 1.4 × longer than apical width; T2–T4 1.6 × longer than apical width ..... ***B. hodorii*** **Kang, sp. nov.**



***Braunsia hodorii* Kang, sp. nov.**
https://zoobank.org/3F31B9BB-546B-4AE0-B50C-2D8C0D192829

Material examined. *Holotype*: Korea • ♀; Gangwon-do, Yeongwol-gun, Munsan-ri, near Jatbong (peak); 37°15.81'N, 128°31.23'E, 229 m a.s.l.; 6 May. 2022 (host caterpillar collection date); S. Kim, I. Park, S. Hong leg.; reared from *C. perspectalis*; host plant: *Buxus* sp.; det. Ilgoo Kang. ***Paratypes*:** • 1♀, 2♂, same as holotype. • 1♂, same as holotype except the host caterpillar collection date, collector and parasitoid eclosion date: 26 May. 2023; S. Kim leg., 1 June. 2023 • 2♂, Gangwon-do, Yeongwol-gun, Bangjeol-ri, near Seondol; 37°12.35'N, 128°26.02'E, 296 m a.s.l.; 10 May 2024 (host caterpillar collection date); I. Kang & S. Kim leg.; reared from *C. perspectalis*; host plant: *Buxus* sp.; det. Ilgoo Kang. Holotype and paratypes will be deposited at NIBR, two paratypes will be deposited at KNUS.

Diagnosis. Members of *Braunsia hodorii* sp. nov. are most similar to those of *B. matsumurai* (Fig. 6) and can be distinguished from them by the characters in the key and from all other members of *Braunsia* by the combination of the following characters: Forewing yellow with two melanic bands (Fig. 2B). Hind wing Cub vein partially present, neither reaching cu-a nor posterior margin; distance between cu-a to Cub closer than the space of *B. matsumurai* (Fig. 2B). Hind coxa and femur mostly melanic. Hind tibia with 7–8 spines apically (Fig. 3B). Median areola of propodeum mostly black, open posteriorly (Fig. 2E). T1 1.4 × longer than apical width, bi-colored, anteriorly pale posteriorly black, with longitudinal carinae in posterior 2/3 (Fig. 2F). T2–T4 1.6 × longer than apical width. T5 striate on anterior third. T6 to remaining tergites yellowish-orange (Fig. 2F).

Description. Holotype. Body 12.6 mm. Forewing length: 11.3 mm. Hind wing length: 8.6 mm.

Head. Antenna with 44 flagellomeres. Interantennal space with weak median carina. POL 1.3 × longer than diameter of anterior ocellus (0.18:0.14). Eye without setae; length of eye 2.3 × longer than median width of gena in lateral view (0.60:0.26). Frontoclypeal suture absent. Malar space 2.8 × longer than basal width of mandible (0.36:0.13). Mandible bidentate; lower tooth acute; upper tooth obtuse. Maxillary palpus five-segmented; apical maxillary palpomere 1.2 × longer than penultimate maxillary palpomere (0.28:0.23). Labial palpus four-segmented. Posterolateral corner of gena sharp.

Mesosoma. Pronotum mostly smooth, anteriorly carinate with crenulate posterior margin. Notauli present, deep and smooth. Scutellar sulcus with round anterior margin, weekly crenulate. Postscutellar depression absent. Propodeum mostly smooth; median areola of propodeum present, closed anteriorly, opened posteriorly; anterior transverse carina of the propodeum reaching lateral margin. Mesopleuron mostly smooth, 1.6 × longer than median width (2.15:1.31), dorsally and posteriorly with crenulate margin; precoxal sulcus present at posterior 3/4. Metapleuron dorsally smooth and posteroventrally strongly sculptured.

Legs. Combined length of foretrochanter and trochantellus 0.5 × longer than foretibia (0.58:1.15). Combined length of mid trochanter and trochantellus 0.3 × longer than basitarsus (0.56:1.86). Mid tibia with 1–2 spines medially and 1–2 spines apically

(variation among females). Hind tibia 1.6 × longer than hind femur (3.63:2.29), with 7–8 spines apically (variation among females); basal spur on hind tibia 0.3 × longer than basitarsus (0.64:1.88). Claws simple and with a basal lobe.

Wings. Forewing second submarginal cell trapezoidal, 1.1 × longer than width (0.40:0.36); RS+M vein of forewing absent; pterostigma 3.5 × longer than medial

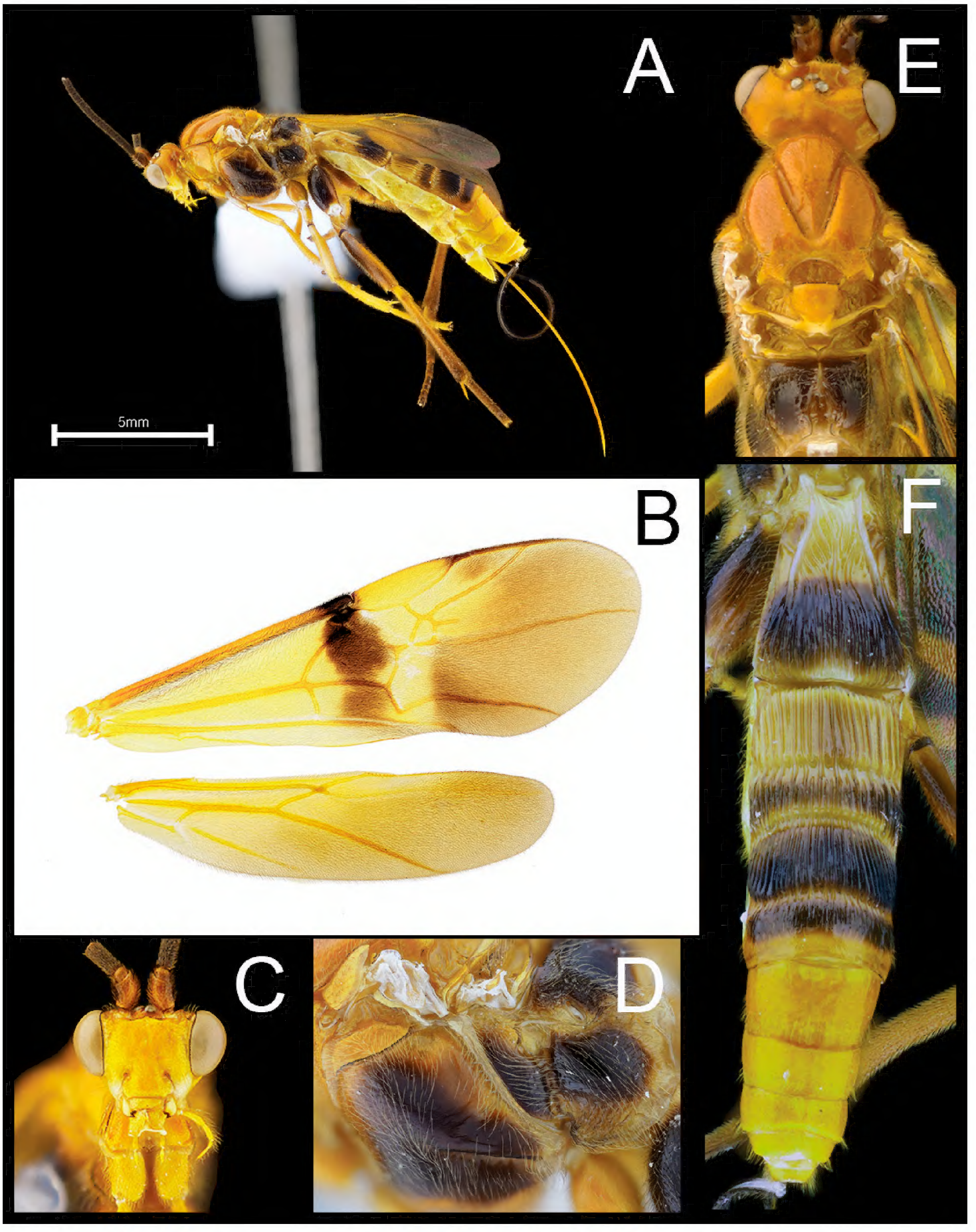


Figure 2. *Braunsia hodorii* sp. nov., holotype, ♀: **A** lateral habitus **B** wings **C** anterior head **D** mesopleuron and metapleuron **E** head and mesosoma, and **F** metasoma. Scale bar: 5 mm (**A**).

width (2.14:0.62); spurious vein, RS2b present, as long as 2RS; 2cu-a absent. Hind wing Cub vein partially present, neither reaching cu-a nor posterior margin; subbasal cell not angled distally.

Metasoma. Metasoma length: 7.4 mm. T1 entirely striate, with horizontal striae at anterior third and longitudinal striae at posterior 2/3; pair of carinae strong, reaching posterior margin; median length of T1 1.4 × longer than apical width (2.22:1.60). T2 1.6 × longer than T3 (0.90:0.58). T2–T4 entirely striate, with longitudinal striae. T5 mostly smooth, with weak longitudinal striae anteriorly. Remaining tergites entirely smooth. Protruded ovipositor as long as metasoma (7.4:7.6), 2.1 × longer than hind tibia (7.6:3.63). Ovipositor length: 7.6 mm.

Male. Similar to female except the following: Body slightly shorter than female, 11.5 mm. Antenna with 42 flagellomeres. Mid tibia with 3 spines medially and 3 spines apically (Fig. 4).

Color. Mostly pale orange and yellow except the following which are brown to black: antenna, mesopleuron, metapleuron, mid coxa, hind coxa, hind femur, hind tibia posteriorly, hind tarsus, hind claw, propodeum, T1 posteriorly, T2 medially, T3 posteriorly, T4, T5 anteriorly. Forewing yellow with two melanic bands. Hind wing yellow basally brown apically.

Distribution. Yeongwol-gun, Gangwon-do, South Korea.

Etymology. The species is named after "Hodori", the male tiger mascot of the Seoul Olympic games in 1988 due to similarity in the color patterns (orange and with black stripes) (Fig. 5). Additionally, the long antennae resemble Hodori's distinctive hat streamer.

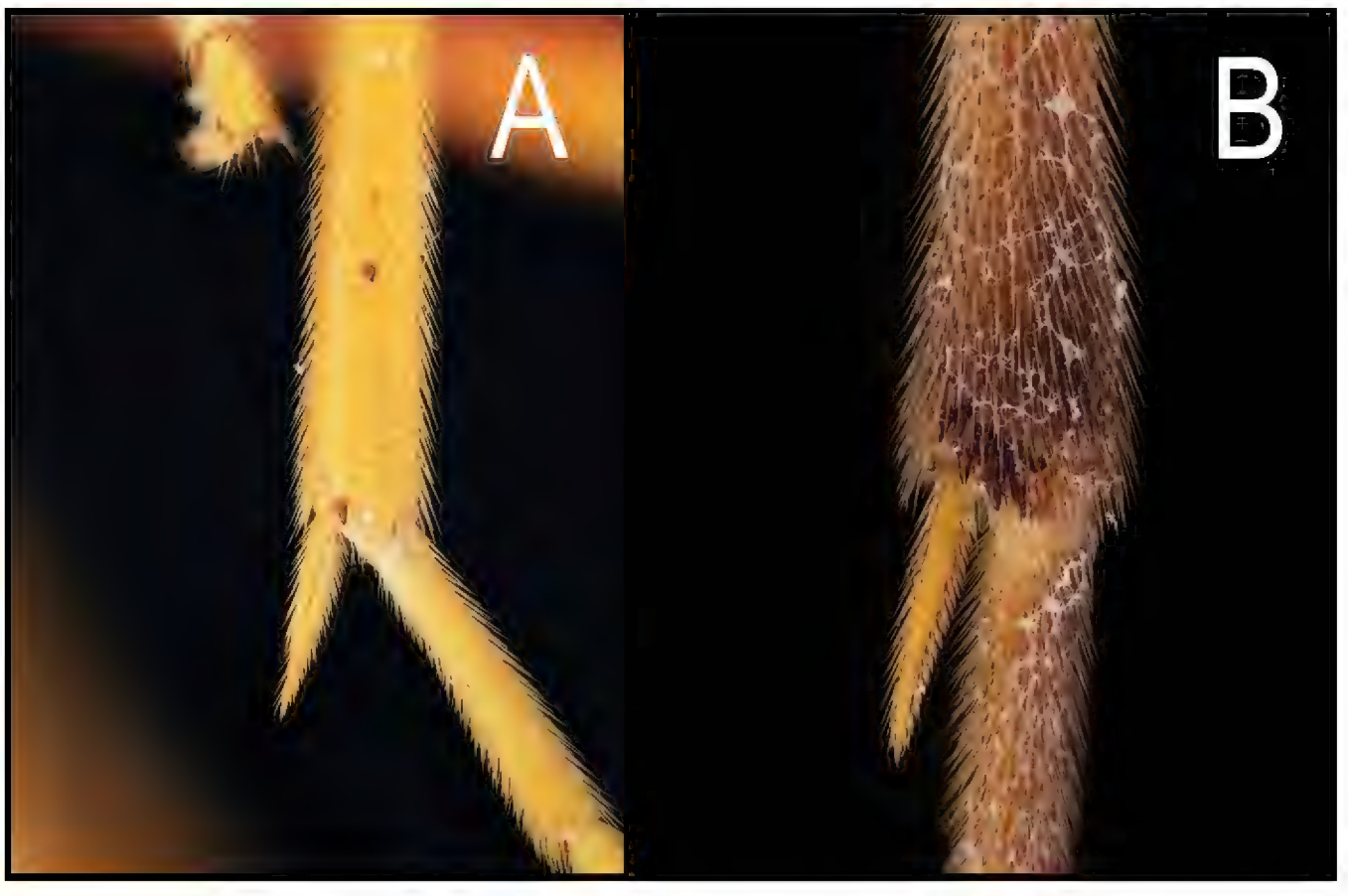


Figure 3. Mid tibia and hind tibia of *B. hodorii* sp. nov., holotype, ♀): **A** spines on mid tibia, and **B** spines on hind tibia.

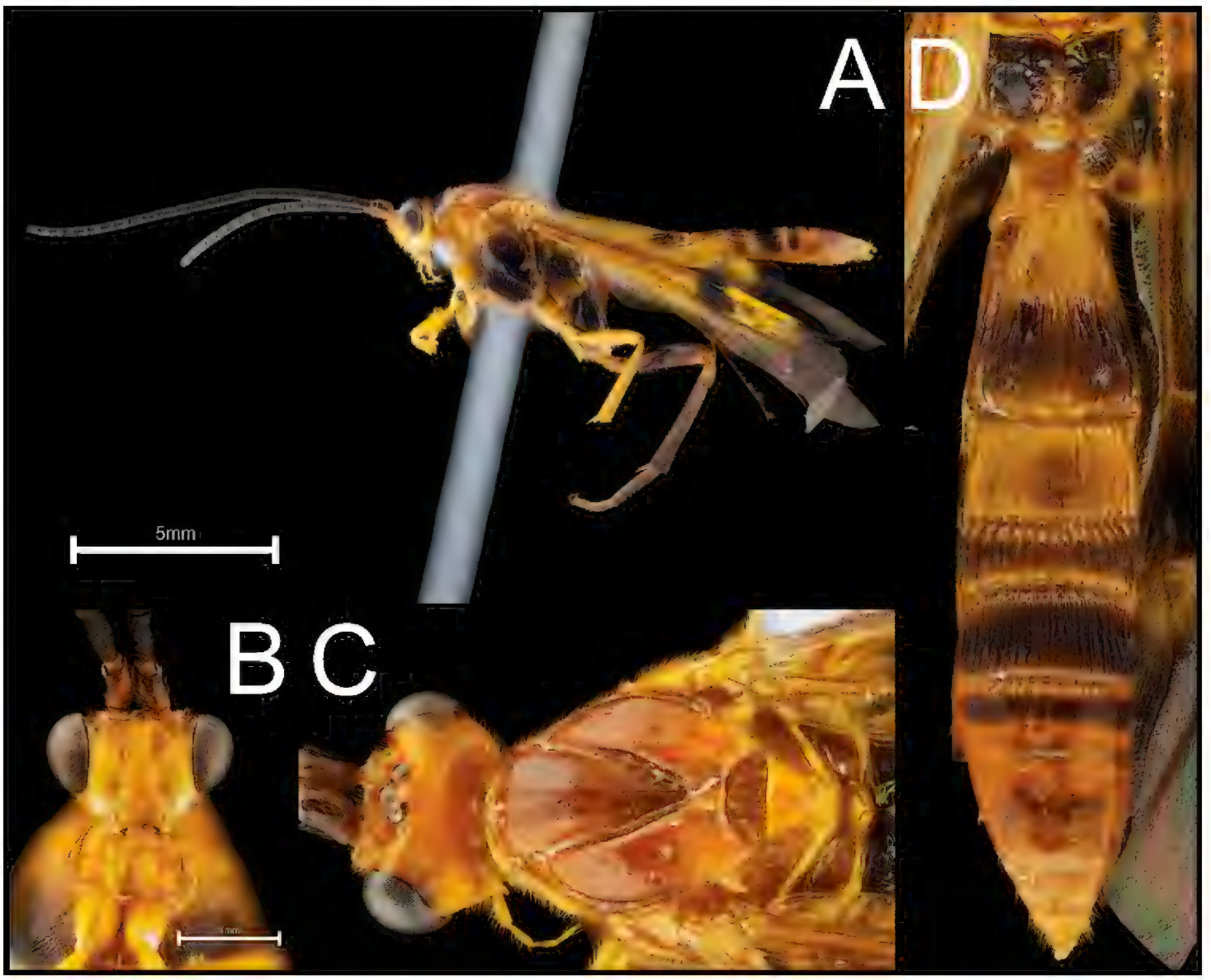


Figure 4. *Braunsia hodorii* sp. nov., paratype, ♂: **A** lateral habitus **B** anterior head **C** head and mesoscutum, and **D** propodeum and metasoma.

Figure 5. Lateral view *B. hodorii* sp. nov. female that was preserved in ethanol.

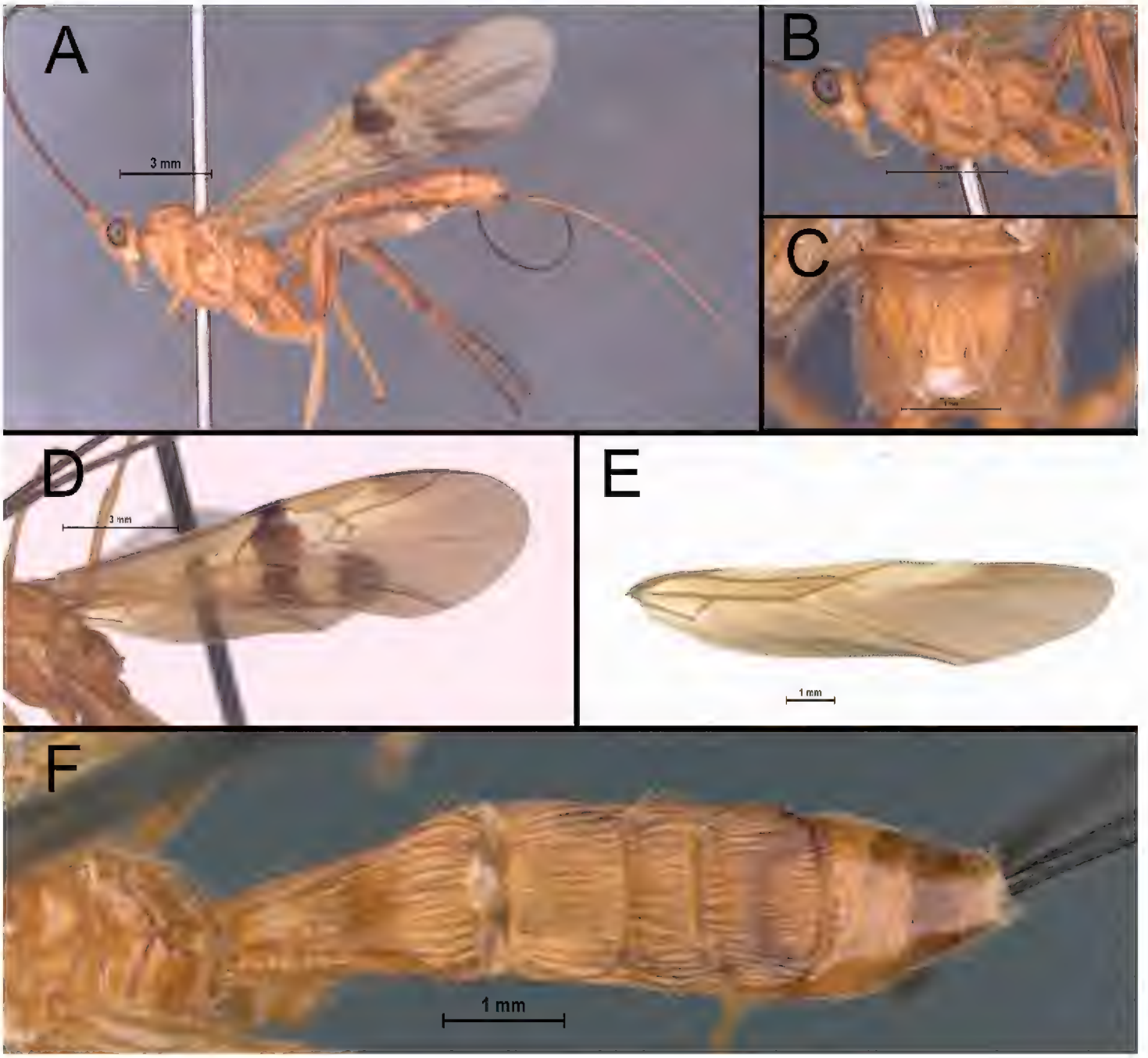


Figure 6. *Braunsia matsumurai* female: **A** lateral habitus **B** lateral head to mesosoma **C** propodeum **D** forewing **E** hind wing, and **F** metasoma.

Notes. Korean common name: 호돌이구멍줄고치벌 (신칭). "호돌이" means the male tiger mascot of the Seoul Olympic Games in Korean and "구멍줄고치벌" is the Korean common name of *Braunsia* spp.

Molecular data. NCBI Accession Numbers: PP437200, PP441967 (COI); PP464023, PP464024(28S).

Host. *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae).

Biology. Larvae of *Cydalima perspectalis* are leaf-webbers, loosely weaving leaves together with silken threads (Fig. 7). Unparasitized, last-instar larvae (6th or 7th instar) typically build a dense shelter with their silk, in which they pupate. Parasitized *C. perspectalis* larvae build highly similar shelters, although somewhat less dense, prematurely, in their 5th instar. They cease movement, and their bodies gradually become shorter and fatter within the shelter before the egression of the parasitoid larva.

Like most other Agathidinae, members of *Braunsia hodorii* sp. nov. are solitary koinobiont endoparasitoids. Upon the egression of parasitoid larvae from the host

Figure 7. A Woven leaves with silken threads spun by *Cydalima perspectalis* larvae **B** parasitoid cocoon within the web of *C. perspectalis*.

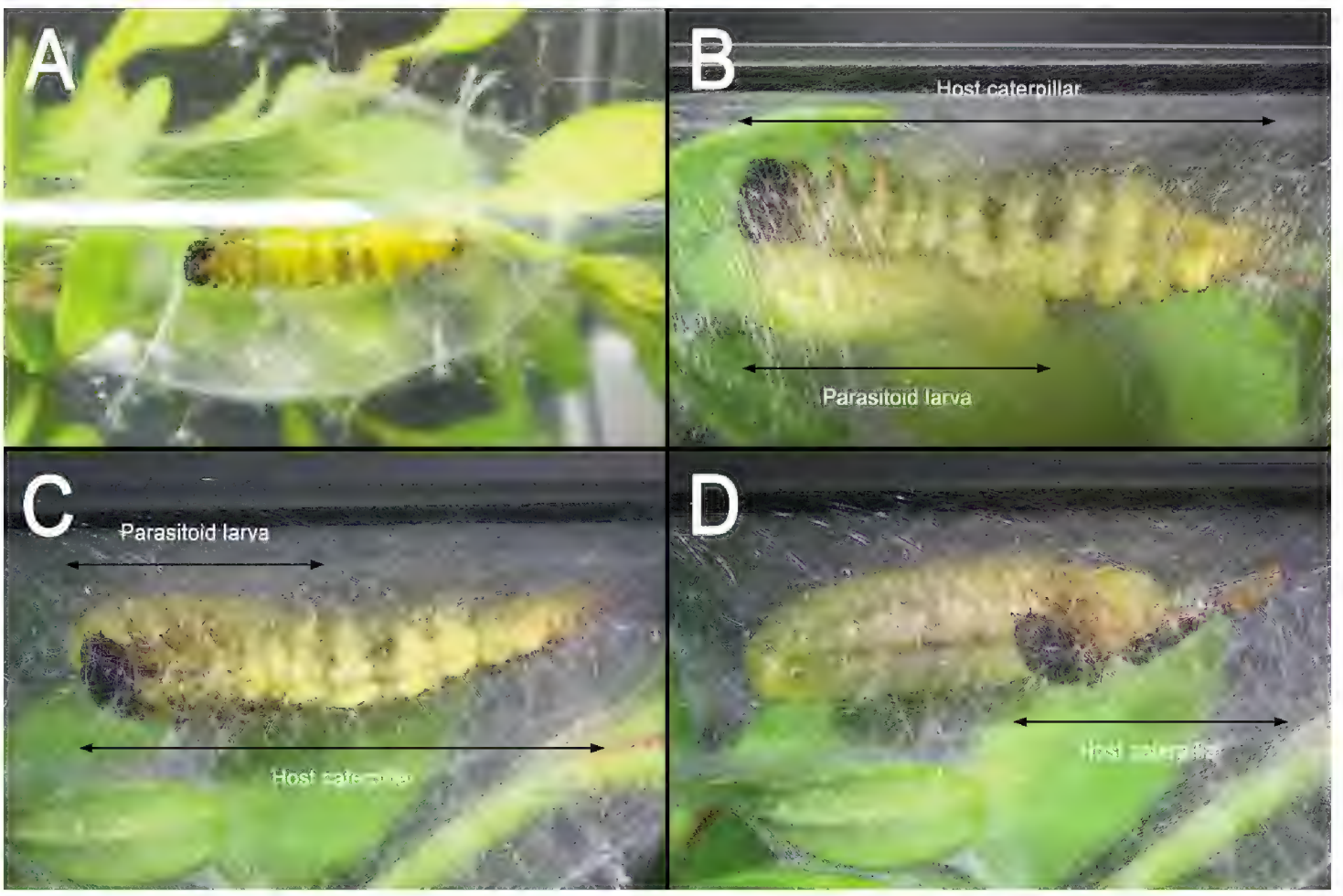


Figure 8. A Parasitized host caterpillar in an inner space made of silk **B, C, D** *B. hodorii* sp. nov. larva consumes the host caterpillar during an external feeding phase.

caterpillars, the parasitoid larvae are greenish-yellow, while the host caterpillars retain their original color. The parasitoid larvae then enter an external feeding phase (Suppl. material 4). The host caterpillars gradually shrink, leaving only their skin and head capsule. Among agathidine braconids, this external feeding phase was only reported for *Therophilus dimidiator* (Nees, 1834) (as *Agathis laticinctus*) (Dondale 1954) before the present study. The external feeding phase takes a little more than 8 hours, with the parasitoid larvae completing their feeding during this period (Fig. 8). Parasitoid larvae then spin a semi-translucent cocoon in the silk web of *C. perspectalis*, where they pupate (Figs 7, 9 and Suppl. material 5). Pupal development lasts about ten days at 25 ± 1 °C.

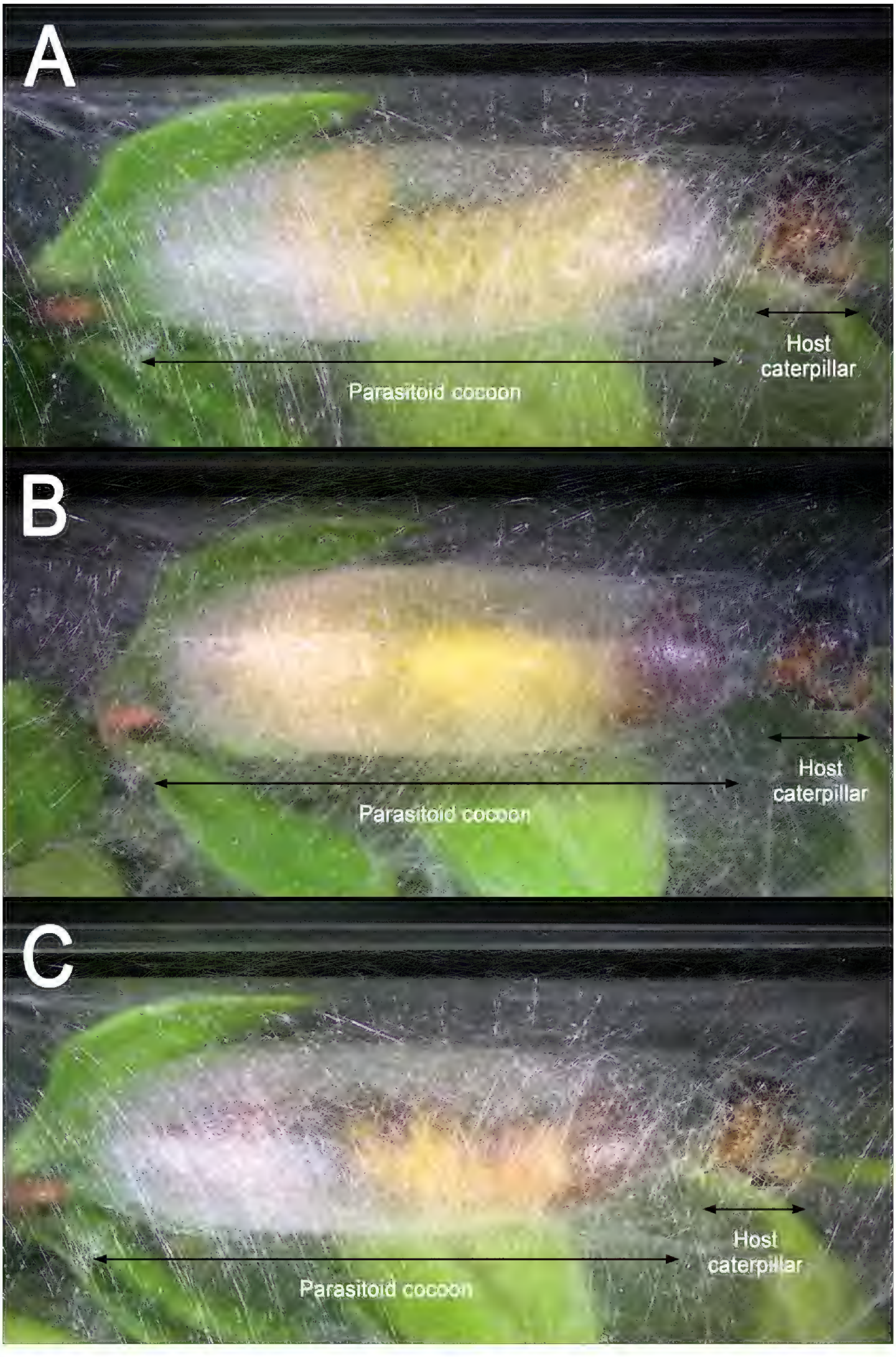


Figure 9. Semi-translucent cocoon of *B. hodorii* sp. nov.: **A** parasitoid larva spinning the cocoon during the first day of cocoon formation **B** parasitoid pupa in the cocoon after six days, and **C** melanizing parasitoid pupa in the cocoon after eight days.

Discussion

Species delimitation and phylogenetic analyses

Initially, the primary goal of the project was to enhance our species concepts based on integrative taxonomic data, morphological data and molecular data, an approach that has been demonstrated in many studies (Jasso-Martínez et al. 2019, 2024; Quicke et al. 2021, 2024; Slater-Baker et al. 2022; van Achterberg et al. 2024; Žikić et al. 2024). We successfully established morphospecies concepts. Unfortunately, due to the age and fungal contamination of the specimens in our possession, we were unable to obtain DNA from exemplars of *B. antefurcalis* and *B. matsumurai*. Given the limited molecular data, we added sequence data of *Braunsia* available in public databases, NCBI GenBank and BOLD, to our data to support our hypotheses. These data allowed estimates of genetic distances based on COI barcodes and we were able to construct phylogenetic trees using 28S and COI sequences.

Based on the genetic distance estimations (Table 2), we confirmed that COI barcoding for *Braunsia* species is a useful tool for delimiting species, given distances among species are relatively high compared to typical COI barcode-based species boundaries, 2.0%, which is also utilized by the BIN algorithm (Ratnasingham and Hebert 2013).

For multigene analyses, we selected taxa represented by both 28S and COI sequence data available in GenBank and BOLD. When either 28S or COI sequence was unavailable, the taxa were excluded. The phylogenetic trees clearly indicate that *B. hodorii* sp. nov. is not conspecific with any other *Braunsia* species included in the study. In addition, two main clades separated by geographic regions suggest that geographic isolation affected diversification of *Braunsia* members (Fig. 1 and Suppl. material 2).

Despite these limitations, this work serves as a starting point for understanding the species boundaries and evolutionary relationships within *Braunsia* based on integrative data. Future research for Korean *Braunsia* should prioritize obtaining fresh specimens and generating complete molecular data for all relevant taxa. Sharkey and Clutts (2011) has been the only published taxonomic research on *Braunsia* based on molecular data prior to the current study. Collaborative projects among braconid taxonomists worldwide are essential to enhance our understanding of poorly understood taxa such as *Braunsia* with broad global distributions.

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Supplementary material 1

The NJ tree based on the concatenated 28S and COI data for *Braunsia* species
Authors: Soohyun Kim, Jong Bong Choi, Hwal-Su Hwang, Marc Kenis, M. Lukas Seehausen, Ikju Park, Jin-Kyung Choi, Kyeong-Yeoll Lee, Michael J. Sharkey, Ilgoo Kang
Data type: png
Explanation note: The bootstrap values are shown along the branches.
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Link: https://doi.org/10.3897/jhr.97.135728.suppl1

Supplementary material 2

The ML tree based on the concatenated 28S and COI data for *Braunsia* species
Authors: Soohyun Kim, Jong Bong Choi, Hwal-Su Hwang, Marc Kenis, M. Lukas Seehausen, Ikju Park, Jin-Kyung Choi, Kyeong-Yeoll Lee, Michael J. Sharkey, Ilgoo Kang
Data type: png
Explanation note: The bootstrap values are shown along the branches.
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Link: https://doi.org/10.3897/jhr.97.135728.suppl2

Supplementary material 3

The NJ tree based on only COI data for *Braunsia* species
Authors: Soohyun Kim, Jong Bong Choi, Hwal-Su Hwang, Marc Kenis, M. Lukas Seehausen, Ikju Park, Jin-Kyung Choi, Kyeong-Yeoll Lee, Michael J. Sharkey, Ilgoo Kang
Data type: png
Explanation note: The bootstrap values are shown along the branches.
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Link: https://doi.org/10.3897/jhr.97.135728.suppl3

Supplementary material 4

Video: feeding behavior of *Braunsia hodorii* sp. nov.

Authors: Soohyun Kim, Jong Bong Choi, Hwal-Su Hwang, Marc Kenis, M. Lukas Seehausen, Ikju Park, Jin-Kyung Choi, Kyeong-Yeoll Lee, Michael J. Sharkey, Ilgoo Kang

Data type: mp4

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Link: https://doi.org/10.3897/jhr.97.135728.suppl4

Supplementary material 5

Video: cocoon spinning by *Braunsia hodorii* sp. nov.

Authors: Soohyun Kim, Jong Bong Choi, Hwal-Su Hwang, Marc Kenis, M. Lukas Seehausen, Ikju Park, Jin-Kyung Choi, Kyeong-Yeoll Lee, Michael J. Sharkey, Ilgoo Kang

Data type: mp4

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